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14. ABSTRACT

After traumatic brain injury (TBI), the human brain sometimes develops tau pathology partly resembling the hallmark neuropathological features of the tauopathy of Alzheimer's disease (AD). Although tau has been strongly linked to the pathogenesis of AD, its involvement in the pathophysiology of TBI and its influence on brain structural and functional outcomes are unclear. Here we used a novel mouse model of early stage AD-type tauopathy to critically evaluate three hypotheses: (i) tau exacerbates the neuronal damage and cognitive dysfunction after single and repetitive mild TBI in the acute and chronic post-injury periods; (ii) mild TBI promotes the severity and spread of tau pathology to contribute to development of a chronic neurodegenerative disorder; and (iii) novel biomarkers for neurodegeneration are non-invasive blood measures of brain dysfunction valuable for the diagnosis and prognosis of mild TBI-triggered chronic neurodegenerative disease.

At the completion of year 3 of the project we conclude that, in both the acute and chronic post-injury time periods, there is no structural or functional evidence for interaction between hippocampal input-specific expression of pathological human tau and either single or repetitive mild TBI. Instead, long-term expression of pathological human tau in a specific mouse neural circuit leads to the onset of tau hyperphosphorylation, aggregation, and slowly progressive neurodegeneration, and this tauopathy occurs independent of single or repetitive mild TBI.

15. SUBJECT TERMS None listed

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Introduction and Overall Objectives

After traumatic brain injury (TBI), the human brain sometimes develops tau pathology partly resembling the tauopathy that is well-established as a hallmark neuropathological feature of Alzheimer's disease (AD). In healthy brain cells, tau is a component of the microtubule network with vital roles in cytoskeletal structure and intracellular transport. However, within vulnerable neurons in signature regions of the AD brain, tau becomes hyperphosphorylated, dissociated from microtubules, aggregated, and mislocalized within cell bodies and proximal dendrites instead of axonal processes, abnormalities that collectively are referred to as tauopathy. There is considerable evidence in AD that tauopathy drives the loss of neurons and synapses underlying the onset and progression of regional brain atrophy and cognitive impairment. Given that AD is a slowly progressive neurodegenerative and cognitive disorder, and TBI induced by inertial forces, concussive blows, or blast will sometimes lead to chronic, progressive brain atrophy and cognitive decline, the question arises whether AD and TBI may share common underlying tau-dependent pathophysiology. At present, although tau is known to accumulate after TBI and become phosphorylated on multiple residues, its pathophysiological importance to brain damage and dysfunction during the acute and chronic post-injury time periods is unknown. From human TBI studies, it is difficult to determine the contribution of tau to progressive brain damage and dysfunction, owing to their dependence on non-invasive or post-mortem histopathological methods. Furthermore, there are currently no simple, validated blood tests for diagnosing at an early and potentially treatable stage the subset of TBI sufferers that go on to develop chronic progressive neurodegenerative disease.

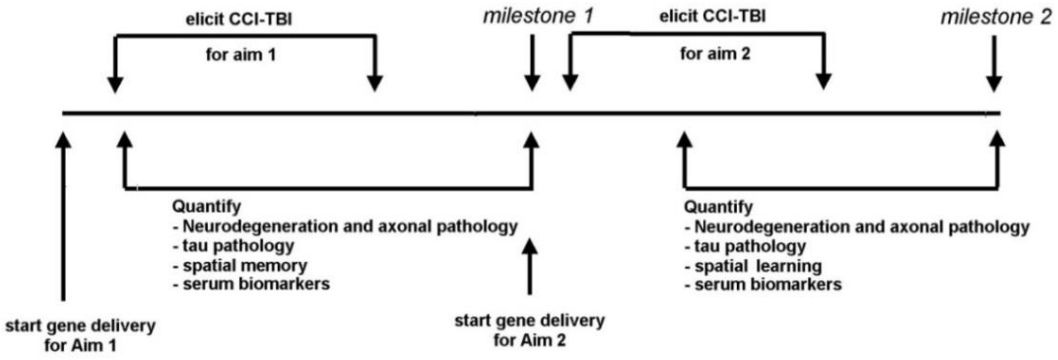
Previously, we established a new translational mouse model for studying pathogenic mechanisms of tau, using a viral vector to drive robust long-lasting expression of a pathological form of mutant human tau focally within a specific hippocampal input pathway that is both preferentially vulnerable in early-stage AD and critically important for long-term memory. The model confines expression of mutant human tau to the lateral perforant pathway, the projection from the lateral entorhinal cortex to the hippocampal dentate gyrus. This mouse model of early-stage AD tauopathy is characterized by rapid, dose-related, circumscribed human tau expression, tauopathy, trans-synaptic spread of human tau expression, and tau-dependent neurodegeneration. The model is exceptionally well suited for addressing whether human tau affects structure and function of the hippocampus after single or repetitive mild TBI, and whether mild TBI exacerbates ongoing tauopathy to promote a chronic neurodegenerative condition. In addition, over the past decade we have discovered and characterized new biofluid-based markers for neurodegeneration. Whereas these novel biomarkers have shown considerable promise as diagnostic and prognostic tools in acute trauma- and ischemia-induced brain injuries, they have never been evaluated as a potential blood diagnostic test for development of a TBI-associated chronic neurodegenerative condition.

Objectives

To study tau/TBI interactions and validate preclinically new biofluid-based diagnostic markers for chronic TBI-induced progressive brain atrophy, we have collaborated with Dr. Douglas Smith, Director of the Penn Center for Brain Injury and Repair, who pioneered the development and characterization of a controlled cortical impact (CCI) model of TBI in the mouse, and our mutual colleague Dr. Victoria Johnson. We combined mild CCI with our novel mouse model of early-stage AD tauopathy to study three critical unanswered questions: (1) does tau exacerbate the acute and chronic effects of mild TBI on brain structure and function? (2) does

mild TBI worsen an ongoing tauopathy to promote development of a chronic neurodegenerative disorder? (3) do blood levels of biomarkers for neurodegeneration diagnose mild TBI cases developing progressive brain atrophy chronically after injury?

Our first two objectives address whether tau is an important target for therapeutic intervention in TBI. Our third objective was to discover and validate pre-clinically a blood test for improving the diagnosis of TBI-induced chronic neurodegenerative disease in the long-term post-injury time period. The time line and milestones for the 3 year project are illustrated below:



Keywords
Tauopathy; tau phosphorylation; traumatic brain injury; concussion; neurodegeneration; entorhinal cortex; perforant pathway; synapse loss; cognitive dysfunction; prognostic biomarker; diagnostic marker; brain atrophy; chronic traumatic encephalopathy; Alzheimer's disease.

Prior Accomplishments: Summary from years 1-2

- Used a novel mouse model of early-stage Alzheimer-type tauopathy, with hippocampal input-specific expression of human tau P301L, or eGFP as a control foreign protein, to evaluate interactions between pathological human tau and controlled cortical impact mild TBI.
- Demonstrated that pathological human tau, expressed specifically in the lateral perforant pathway hippocampal input, does not endanger the neurons, axons, or synapses of the pathway to either single or repetitive mild TBI acutely (within 7 days) post-injury.
- Found that neither single nor repetitive mild TBI changes the expression level, distribution, or phosphorylation of human tau acutely post-injury.
- Showed that both single and repetitive mild TBI impair hippocampus-dependent spatial learning acutely post-injury, and pathological human tau does not exacerbate this acute cognitive dysfunction.
- Demonstrated that at 7 days post-injury, serum levels of SNTF, a mechanism-based biomarker for injury-induced axonal damage, are not changed appreciably after single or repetitive mild TBI, or affected by pathway-specific expression of pathological human tau.
- Launched the second phase of the project, focused on interactions between tauopathy and mild TBI in the long-term (4 month) post-injury period; mice were genetically modified to express mutant human tau and subjected to single or double mild TBI, or sham injury, and readied for qualitative and quantitative analyses of the long-term effects of pathological tau on hippocampal structure and function after single or repetitive mild TBI.

Current Accomplishments: Summary from Year 3

- Established that from 7 days to 4 months after single or double mild TBI, the cortical and hippocampal damage continues to worsen; this evolving brain atrophy occurs irrespective of the presence of mutant human tau in the perforant pathway.
- From an array of structural and functional assessments, there is no appreciable interaction between pathological human tau and either single or double mild TBI chronically post-injury.
- Instead, the viral vector-based model of input-specific mutant human tau expression exhibits slowly progressive tau pathology and the onset of perforant pathway degeneration, occurring independently of mild TBI.
- Tau phosphorylation at residues 212, 214, 217, and 262, aggregation and packaging into presumptive granulovacuolar degeneration bodies are associated with the onset of perforant pathway degeneration, thereby identifying specific post-translational modifications and a cell biological process that are potentially important triggers for slowly progressive tau neurotoxicity in chronic neurodegenerative disease.
- Created a serum bank from genetically modified mice sampled 4 months after sham injury, single or double mTBI that were characterized for cognitive and histopathological changes chronically post-injury. In order to complete analyses of candidate diagnostic surrogate markers for TBI-induced chronic brain damage and quantitative analysis of synapse integrity, the project received a 6 month Extension Without Funding to March 29, 2018.

Accomplishments and Major Findings from Year 3

1. <u>The novel mouse model of early-stage Alzheimer-type tauopathy</u>

There is considerable evidence that dysfunction of the perforant pathway projection from entorhinal cortex (EC) to hippocampal dentate gyrus is an important contributor to the onset and progression of cognitive impairment in AD. This pathway is a major source for excitatory

innervation of hippocampus, a structure vital for memory formation. Damage to the EC or perforant pathway projection in animals causes a rapid forgetting syndrome reminiscent of AD. The perforant pathway is especially vulnerable in AD. The entorhinal neurons of origin in layer II are among the first to develop aggregates of hyperphosphorylated tau in the form of neurofibrillary tangles (Braak stage I), and the terminal field in the dentate gyrus is a preferential early site for amyloid Aβ deposition. Recent evidence suggests that tauopathy initiating in the perforant pathway spreads over time through its afferent connections. Finally, the pathway dies beginning with the earliest signs of cognitive impairment, and the neuronal loss progresses coincident with cognitive decline, until more than 90% of the pathway has degenerated.

Consequently, we used an AAV vector approach to express pathological human mutant tauP301L linked genetically to human tauopathies, or an eGFP control, focally in the mouse lateral perforant pathway. The vectors were microinjected by unilateral stereotaxic convection-enhanced delivery into the right lateral entorhinal cortex (EC), and 4 weeks allowed for the foreign proteins to be expressed in the entorhinal layer II neurons of origin, the perforant pathway axons, and their synapses onto the distal dendrites of granule neurons in the dentate gyrus outer molecular layer (OML). To set the stage for the histopathological studies of chronic interactions between mild TBI (mTBI) and pathological tau conducted in year 3 and the focus of this report, we briefly review the anatomy of the mouse perforant pathway input to hippocampus and features of the AAV-based mouse tauopathy model. As schematized in Figure 1, in the mouse all of the synaptic inputs terminating in the OML originate from the lateral EC and lateral perforant pathway projection.

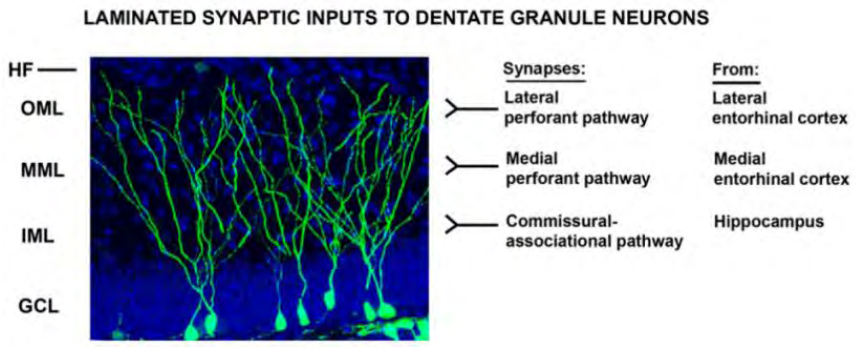


Figure 1 abbreviations used:
HF- hippocampal fissure
OML – outer molecular layer
MML – middle molecular layer
IML – inner molecular layer
GCL – granule cell layer
A subset of granule neurons and their dendrites are shown in green, nuclei are in blue.

Confirming our earlier publications (Siman et al., 2013; 2015), delivery of AAV-hTauP301L to the right lateral EC led to robust human tau expression in the lateral peforant pathway EC layer II neurons of origin and the entire projection as it traverses the stratum lacunosum-moleculare (SLM) of hippocampal CA1 sector before perforating the hippocampal fissure (HF) to terminate in the dentate OML (see Annual Report, 2015). The human tau is distinguished from the widely distributed endogenous mouse protein by immunohistochemistry using the human specific monoclonal HT7. In contrast, tau phosphorylated on serine residues 202 and 205, labeled with the monoclonal AT8 and considered an early marker for hyperphosphorylation, is confined to layer II neurons of the lateral EC, but does not undergo appreciable axonal transport and is undetectable in the perforant pathway axons or synapses. This distribution of pTau202/205 closely resembles the earliest neuropathological stage of Alzheimer tauopathy (Braak stage I), with hyperphosphorylated tau aggregates localized to the superficial trans-entorhinal region. An identical distribution pattern is observed for tau phosphorylated on Thr231 or Ser262 at 4-5 weeks after gene delivery. With AAV-eGFP as a

control for vector-mediated foreign protein expression, the autofluorescent eGFP distributes similarly to human tau throughout the lateral perforant pathway neurons of origin, axons, and pre-synaptic terminals innervating the hippocampal dentate gyrus and terminating in the OML.

2. Chronic interactions between mTBI and tauopathy studied in project year 3

Our 3 year project addressed three key questions in the search for tau/mTBI interactions in the acute and chronic post-injury periods:

(1) What are the effects of pathological hTau on the response to mTBI at 7 days and 4 months post-injury?
(2) Does mTBI exacerbate tau pathology or promote its anatomical spread either acutely or chronically post-injury?
(3) Is there a blood biomarker for neurodegeneration that in the long-term post-injury period serves as a surrogate marker for chronic mTBI-induced brain damage?

To identify mTBI-induced brain atrophy and any changes in tau pathology that may have developed only in the long-term post-injury period, we compared genetically modified mice at 4 months post-injury (chronic phase) with mice at 7 days post-injury (acute phase). For the acute component of this study, we analyzed the following groups of mice at 7 days post-injury, 5 weeks after viral vector-driven transgene expression:

1) AAV-eGFP, sham injury (n=10)
2) AAV-hTau, sham injury (n=15)
3) AAV-eGFP, mild TBI (n=11)
4) AAV-hTau, mild TBI (n=9)
5) AAV-eGFP, double mild TBI (n=9)
6) AAV-hTau, double mild TBI (n=10)

For the chronic component of the study, comparably treated mice were analyzed at 4 months post-injury (5 months after viral vector-driven transgene expression):

1) AAV-eGFP, sham injury (n=7)
2) AAV-hTau, sham injury (n=13)
3) AAV-eGFP, mild TBI (n=8)
4) AAV-hTau, mild TBI (n=14)
5) AAV-eGFP, double mild TBI (n=10)
6) AAV-hTau, double mild TBI (n=11)

Our experimental design yielded sufficient numbers of each experimental group for drawing definitive conclusions on the experimental questions posed above. Of the mice intended for the acute study component, 64 across the 6 treatment groups were confirmed by blinded histological analysis to exhibit strong and highly localized eGFP or hTau transgene expression throughout the entire rostro-caudal extent of the lateral perforant pathway. For the chronic study component, an additional 63 mice were confirmed as maintaining strong and long-lasting transgene expression throughout the pathway.

At either 1 or 5 months after gene delivery, and either 7 days or 4 months after injury, the mice were evaluated for the following hippocampal structural and functional endpoints:

(a) human tau expression (human-specific tau antibody HT7)
(b) tau phosphorylation (pTau202/205; pTau231; pTau262; pTau212/214/217)
(c) human tau trans-synaptic propagation (human-specific tau antibody HT7)
(d) perforant pathway neuronal survival (NeuN antibody)

(e) perforant pathway synapse and axonal integrity (synaptic zinc using Timm's stain)
(f) perforant pathway axonal pathology (APP and SNTF antibodies)
(g) hippocampus-dependent spatial learning (Morris Water Maze over 3 consecutive days)

In addition, serum samples were obtained at the time of sacrifice at 7 days or 4 months post-injury for quantification of the calpain-derived spectrin fragment SNTF (nonerythroid α-spectrin 1-1176) as a persistently elevated blood biomarker for mTBI. During year 2, we completed all of the short-term behavioral, histological (both qualitative and quantitative), and serum biomarker analyses for the mice with well-place vector injections and robust lateral perforant pathway transgene expression. This established the foundation to search for progressive neurodegenerative changes or tauopathies that might develop only in the long-term period after mTBI. The following sections describe our findings and summarize conclusions on the influence of pathological hTau on the long-term outcomes from single and double mTBI, and the long-term influence of single and double mTBI on the severity and distribution of tau abnormalities in our novel mouse model of early-stage Alzheimer tauopathy.

3. A mild controlled cortical impact TBI in the mouse elicits subtle hippocampal structural damage on the impacted side acutely post-injury that evolves for at least 4 months post-injury.

In order to examine the effect of long-term expression of pathological tau in the perforant pathway, we first evaluated eGFP and hTau expression at 5 months after gene delivery. As shown in Figure 2 and later in Figure 4, robust and focal expression of both pathological human tau and eGFP persisted for at least 5 months throughout the lateral perforant pathway, originating in layer II of the lateral EC (left panels), projecting through the hippocampal SLM, and terminating in the dentate OML (right panels).

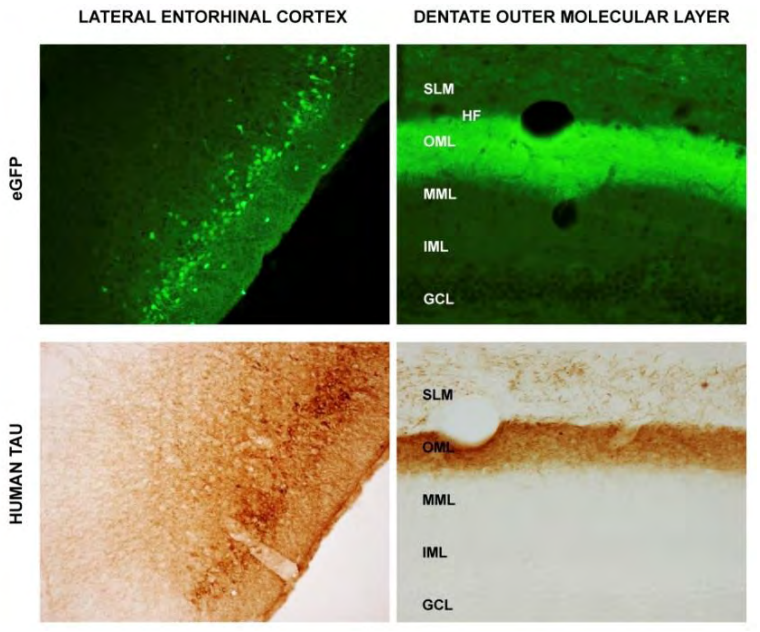


Figure 2. Strong transgene expression persists for at least 5 months in the lateral perforant pathway after AAV-mediated gene delivery. (Left panels) – Lateral entorhinal cortex. The lateral perforant pathway neurons in layer II continue to strongly express either eGFP or hTauP301L. (Right panels) – Hippocampus. eGFP and hTau persist in the perforant pathway axons traversing the SLM in the CA1 sector, and in the synaptic field in the dentate outer molecular layer (OML). Other hippocampal regions are devoid of foreign protein expression.

One of our main objectives is to examine effects of mTBI on the structural and functional integrity of a major hippocampal input pathway when it expresses a pathological form of human tau. Consequently, it is vital for our study design that the chosen method for inducing mTBI

elicits discernable but minor hippocampal structural damage. To accomplish this, the magnitude of the controlled cortical impact was adjusted by varying the impounder velocity and cortical depth, so as to produce subtle but discernable hippocampal structural damage.

Having now examined 63 genetically modified sham-injured, single-injured and double-injured mice at 4 months after injury, and in years 1 and 2 of the project another 64 mice at 7 days post-injury, we conclude that cortical lesions at the site of impact are larger at 4 months than at 7 days post-injury, and the distortions of hippocampal cytoarchitechture underlying the site of cortical impact are more prominent. For example, as shown in Figure 3, beneath the impacted lesioned parietal cortex, the hippocampus is mostly intact at 7 days post-injury, as revealed by immunostaining for the neuronal nuclear marker NeuN (compare the top left and bottom left panels). However, hippocampal structure changes post-injury. First, the hippocampus exhibits localized swelling and disrupted cellular organization in the CA1/CA3 sector border beneath the site of controlled cortical impact compared with the uninjured contralateral side (arrows; compare the injured cases with the sham control at the top left). Secondly, the shape of the dentate gyrus granule cell layer (GC) is often distorted and the entirety of the dentate is often compacted.

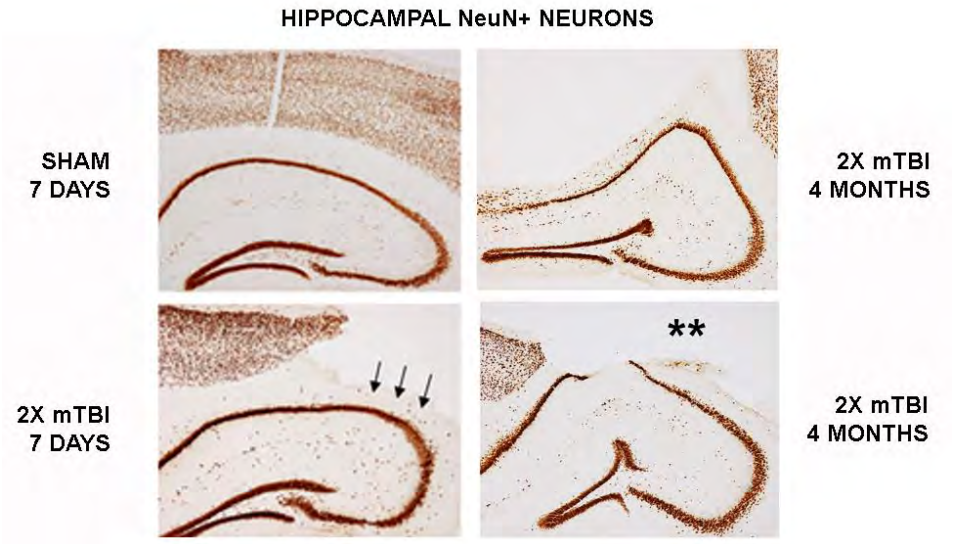


Figure 3. Hippocampal structural damage progressively worsens from 7 days to 4 months after mTBI.
Immunostaining for the neuronal marker NeuN in mouse brain shows the presence of a large focal lesion in parietal cortex at the site of impact (e.g., bottom right panel, double asterisks). In comparison with the sham-injured left hippocampus (top left panel), the CA1 sector is swollen and the cytoarchitecture of the pyramidal cell layer compressed and distorted (arrows) after a double mTBI (bottom left, at 7 days; top right, at 4 months). The same alterations occur after a single injury (shown in previous reports). In a subset of mice, the primary focal lesion extends into the dorsal CA1 sector at 4 months post-injury, and the organization of granule neurons in the dentate gyrus is both disrupted and compacted (right two panels). The hippocampal CA1 swelling and disruption in CA1 and dentate cytoarchitecture are consistently more pronounced at 4 months (right) than at 7 days (left) post-injury for both single and repetitive mTBI.

All of these structural abnormalities are more pronounced at 4 months post-injury. Over time, the primary focal lesion at the cortical site of impact expands, sometimes to include the dorsal part of the rostral hippocampus (lower right panel). The swelling-induced distortion of

the CA1/CA3 sector (top right) and the compression and disrupted organization of the dentate granule cell layer (both right panels) become more pronounced. The cortical and hippocampal structural damage is comparable between single and double mTBI, and does not appreciably differ depending on whether eGFP or hTauP301L is expressed in the lateral perforant pathway hippocampal afferent projection. These data confirm that the controlled cortical impact mTBI used in our study spares the hippocampus from profound atrophy and degeneration, but distorts its structure, including altering the dentate gyrus that contains the lateral perforant pathway axons and synapses, along with the granule neuron targets of the pathway.

Immunostaining for SNTF, established by my laboratory over the past 20 years as a marker for necrotic neurodegeneration, supports the observations described above for the evolution of brain injury chronically post-injury. At 4 months after mTBI, occasional neurons are SNTF-immunopositive in the injured parietal cortex adjacent to the controlled cortical impact site, suggesting they are undergoing frank neurodegeneration (Figure 4). Some of the neurons positive for SNTF have abnormal rounded perikaryal morphology and beaded dendritic varicosities, suggestive of ongoing degeneration (right panel, arrow). On the other hand, despite showing disrupted cytoarchitechture neither the hippocampal CA1 sector nor the dentate granule cell layer show any evidence for SNTF-positive actively degenerating neurons at 4 months post-injury. Similarly, the lateral entorhinal cortex containing the neurons of origin for the lateral perforant pathway in layer II is also devoid of SNTF-positive degenerating neurons.

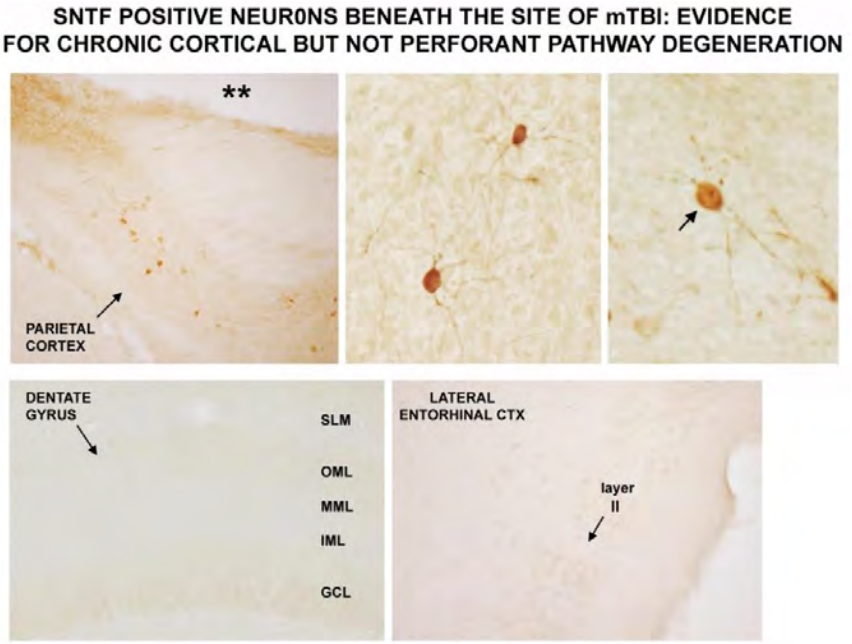


Figure 4. Evidence for ongoing cortical neuronal degeneration near the site of impact but not in the perforant pathway or dentate gyrus target neurons at 4 months after mTBI.

Immunostaining for SNTF, a marker for necrotic neurodegeneration, identifies scattered cortical neurons adjacent to the site of controlled cortical impact 4 months after mTBI. Top row: beneath the impact site (asterisks), scattered neurons are SNTF immunopositive, some of which also have abnormal morphology consistent with ongoing neurodegeneration (right, arrow). In contrast, neither the dentate gyrus (bottom left) or entorhinal cortex (bottom right) contain any acutely degenerating SNTF positive cells at 4 months post-injury, including the layer II perforant pathway neurons and their target neurons in the dentate granule cell layer.

4. Neither short- nor long-term expression of eGFP as a control foreign protein promote degeneration of the perforant pathway after TBI.

We reported in the year 2 Annual Report that combining AAV-driven expression of eGFP in the perforant pathway with single or double mTBI did not induce any loss of pathway neurons, axons, and synapses in the acute post-injury period. Similar results were obtained at 4 months after mTBI (Figure 5, left panels). In the hippocampus, staining for pre-synaptic terminal zinc is a pathway-specific method for analyzing the integrity of afferent inputs arising from multiple brain regions, including from the lateral entorhinal cortex via the lateral perforant pathway. For the eGFP expressing controls, zinc staining of lateral perforant pathway synapses in the dentate outer molecular layer is unchanged at 4 months after mTBI, as observed after both single (middle left) and double injury (data not shown). The lack of toxicity of eGFP occurs despite strong expression of the protein in the lateral perforant pathway axons in the hippocampal SLM and synaptic field in the dentate outer molecular layer (top left). Similarly, at 4 months post-injury there is no overt loss of eGFP expressing lateral perforant pathway neurons of origin in layer II of the lateral entorhinal cortex, compared with the uninjured contralateral side and based on quantitative analysis of NeuN-immunopositive neuronal nuclei (Table 1). These data established our capacity to determine whether expression of human tauP301L in the pathway in lieu of eGFP endangered these neurons and synapses to the chronic effects of mTBI.

5. Expression of pathological human tau does not markedly enhance perforant pathway vulnerability to single or repetitive mTBI in the chronic post-injury period.

We studied whether expression of pathological human tau in the lateral perforant pathway, at a level below the threshold for direct and rapid hTauP301L neurotoxicity, might sensitize the pathway to degeneration chronically in response to single or double mTBI. To investigate this possibility, we conducted analyses using compartment-specific markers for the survival of lateral perforant pathway neurons and of the integrity of its synapses. The survival of lateral perforant pathway neurons in layer II of entorhinal cortex was quantified from a total of 63 mice at 4 months post-injury, 25 expressing eGFP and 38 hTauP301L, using NeuN as a neuronal nuclear marker. To assess synapse integrity for the pathway, zinc staining of lateral perforant pathway presynaptic terminals was used to demarcate the terminal field in the dentate gyrus OML. In the 6 month EWOF period extended to March 29, 2018, we will complete the quantitative analysis of perforant pathway synapse integrity in all 63 long-term post-injury cases.

Our results thus far support and extend the interim findings reported previously indicating a lack of ongoing neurodegeneration in the perforant pathway axonal projection and synaptic field chronically after single or double mTBI. Neuronal integrity of the lateral perforant pathway at 4 months after single mTBI is illustrated in the bottom panels of Figure 4. For the majority of injured mice, with expression of either eGFP (bottom left) or hTauP301L (bottom right) there is no appreciable difference in perforant pathway neuronal density between the injured and uninjured sides. These qualitative observations are substantiated by quantitative analysis of NeuN-positive neuronal density in lateral entorhinal cortex layer II at 4 months post-injury (Table 1). At this long-term time point, neither genotype shows robust neuronal loss in sham-, single-, or double-injured mice. Furthermore, there is no difference in survival of lateral perforant pathway neurons between mice expressing eGFP and pathological human tau in any of the 3 injury groups. In mice with long-term expression of hTauP301L, there is an 11% decrease in lateral perforant pathway neuronal survival at 4 months after double mTBI, and this small effect is statistically significant ($p=0.02$).

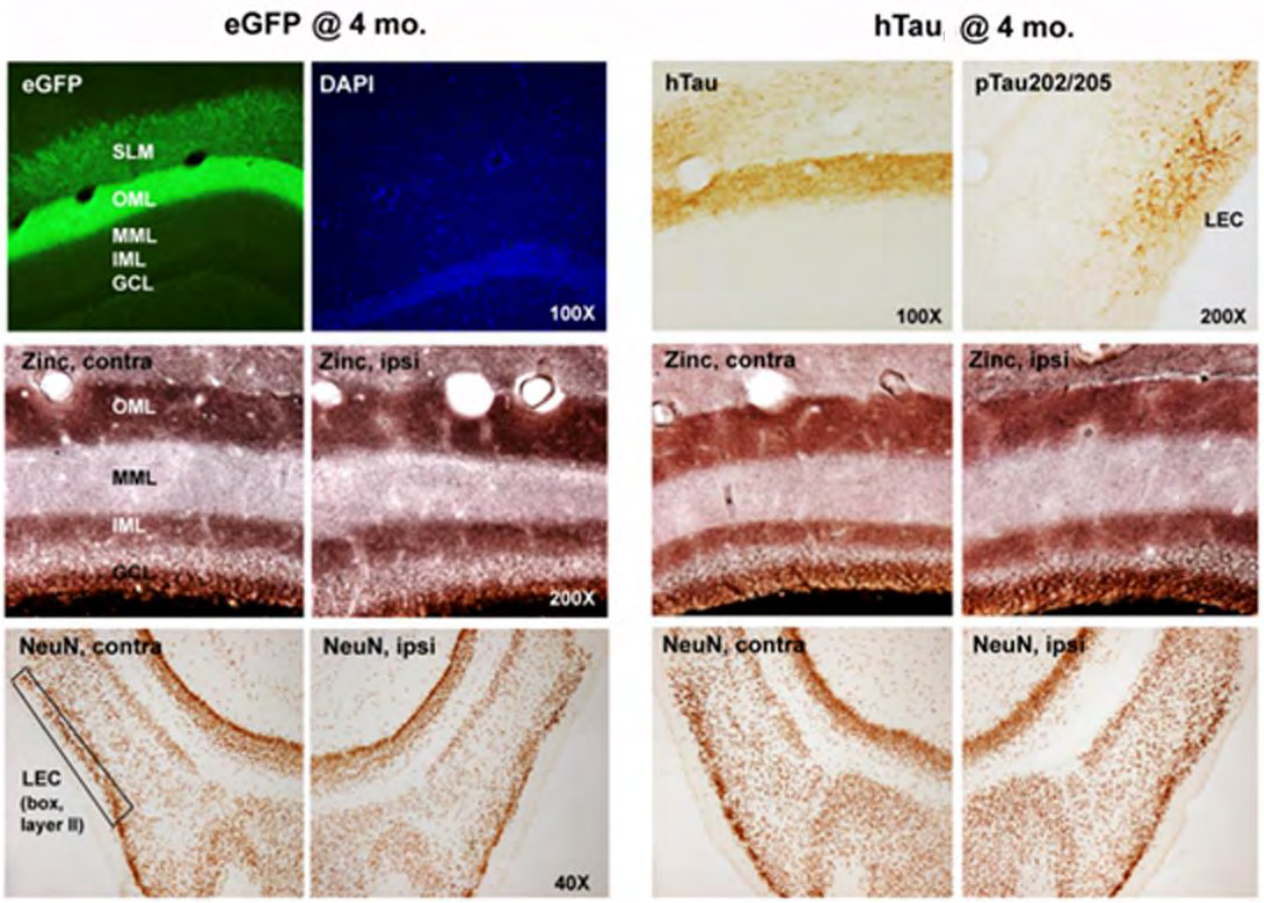


Figure 5. Evaluation of transgene expression, synapse and neuronal integrity after 5 months of eGFP and hTau expression and 4 months after mTBI.
Top panels: Expression of eGFP (left) and hTau (right) are sustained at 5 months after AAV-mediated gene delivery and 4 months after mTBI. Note the robust expression of both proteins in the lateral perforant pathway terminal field in the dentate OML. Also, note that at 5 months after hTau gene delivery, pTau202/205 is abundant in the neurons of origin for the pathway in layer II of the lateral entorhinal cortex (LEC; top right), but undetectable in axons and synaptic terminals of the pathway in the dentate OML, identical to the distribution of pTau acutely after mTBI reported before.
Middle panels: Lateral perforant pathway synapses in the dentate OML appear intact at 4 months after single mTBI coupled with eGFP or hTauP301L expression, as evidenced by the persistence of pre-synaptic terminal zinc staining.
Bottom panels: Lateral perforant pathway neurons in the entorhinal cortex appear intact at 4 months after single mTBI, and after 5 months expression of either eGFP or hTauP301L, as evidenced by the persistence of neuronal nuclei in layer II (denoted by the box).

We conclude that, in the controlled cortical impact mouse model, there is little appreciable interaction between pathological human tau and either single or repetitive mTBI to influence perforant pathway vulnerability in the chronic post-injury time period. The lack of a robust effect of controlled cortical impact mTBI on integrity of the lateral perforant pathway neurons, axons and synapses is not due to failure of the single and double injuries to reach the hippocampus and affect its structure. As described in Section 3 above and illustrated in Figure 2, there are readily discernable abnormalities in the structure of the hippocampal formation and

dentate gyrus at 4 months post-injury that are even more pronounced than at 7 days after mTBI, in the form of disrupted cytoarchitecture, compression of the dentate gyrus, and in some cases circumscribed degeneration of hippocampal pyramidal neurons.

Table 1. Quantitative morphometric analysis of the long-term effect of mTBI on neuronal integrity of the genetically modified lateral perforant pathway. Lateral perforant pathway neuronal survival at 4 months post-injury is represented as the mean NeuN-positive density of layer II neurons on the injured side relative to the control hemisphere, determined for each brain section. P value – unpaired t-test. NS – not significantly different from the sham control groups.

Transgene (n)	Injury	ipsi/contra mean (sem)	P value
eGFP (6)	sham	0.98 (0.03)	
eGFP (9)	mTBI	1.04 (0.05)	NS
eGFP (8)	2x mTBI	1.04 (0.04)	NS
hTau (13)	sham	1.00 (0.04)	
hTau (13)	mTBI	1.05 (0.05)	NS
hTau (11)	**2x mTBI**	**0.89 (0.04)**	0.059 vs hTau sham; **0.02 vs. eGFP 2x mTBI**

6. Our methods are readily capable of detecting perforant pathway neurodegeneration and tau propagation, had they been triggered by mTBI and exacerbated by pathological tau.

To confirm that our analytical methods are capable of detecting partial degeneration of the lateral perforant pathway, should it occur after mTBI combined with expression of hTauP301L, we evaluated the integrity of the pathway after administering a dose of AAV-hTau shown previously to induce partial degeneration even in the absence of TBI. All of the experiments described above used intra-entorhinal delivery of 0.5 billion genome copies of the AAV-hTauP301L or AAV-eGFP vectors. This is the maximal dose of the hTau vector that drives robust human tau expression and hyperphosphorylation without causing a loss of perforant pathway neurons or degeneration of axons or synapses for up to 3 months of expression. We also examined the effects of expressing a higher dose of pathological hTau (1.5 billion genome copies) on perforant pathway integrity and human tau distribution. At this dose, exemplified in Figure 6, human tau is found not only in the perforant pathway axons of the hippocampal SLM and lateral perforant pathway pre-synaptic terminals of the dentate OML, but its expression expands trans-synaptically to include scattered lateral perforant pathway target neurons, the dentate granule cells in the GCL (Figure 6, top left). The trans-synaptic transfer of hTau expression in our AAV model occurs only in association with hTau-triggered partial degeneration of the lateral perforant pathway, and is mitigated by a pharmacotherapy that partially blocks the neurotoxicity of hTauP301L (Siman et al., PLoS One 10: e0142340 [2015]). Direct evidence that pathological human tau expression triggers dose-dependent lateral perforant pathway degeneration is shown by the reduced density of synaptic zinc staining and thinning of the dentate OML (middle panels), and reduced number of NeuN-stained nuclei in layer II of the lateral EC (bottom panels). These results, coupled with our prior published findings (J Neuropathol Exp Neurol 72: 1062 [2013]), demonstrate that the zinc and NeuN labeling methods are capable of detecting partial degeneration of lateral perforant pathway axons, synapses, and neurons, had it been triggered by hTau either acutely or chronically after single or double mTBI.

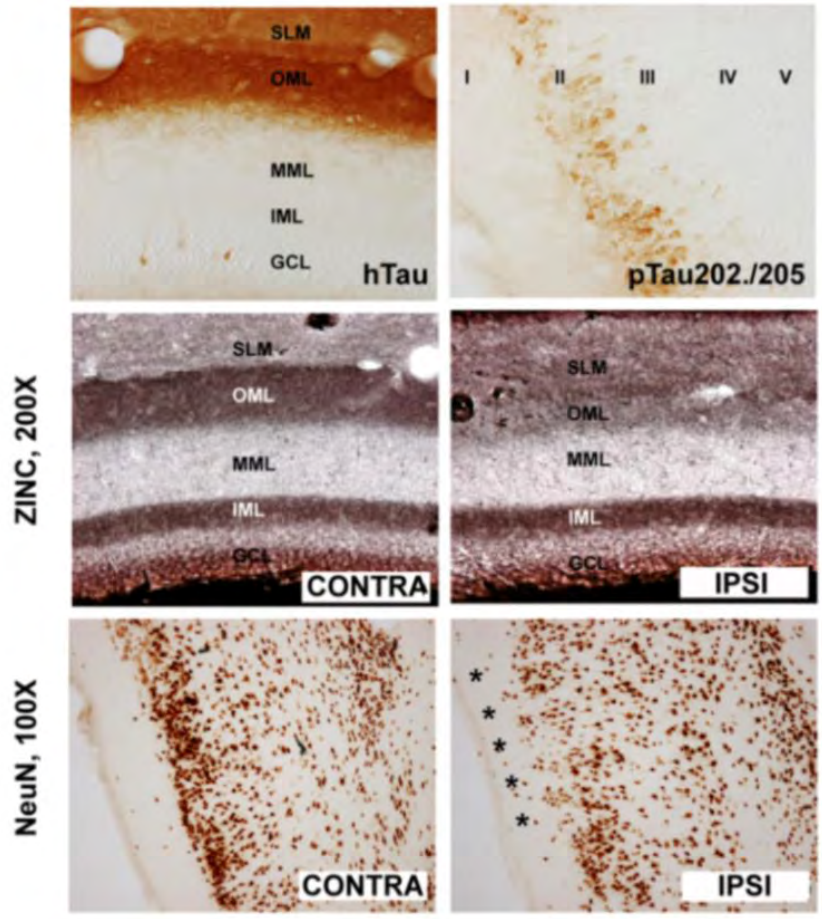


Figure 6. Detection of partial loss of perforant pathway neurons and synapses after delivery of a higher, toxic dose of AAV-hTauP301L. Top panels – Human tau in dentate gyrus and pTau202/205 in lateral EC layer II 5 weeks after delivery of 0.75 billion genome copies, a dose of hTauP301L that causes partial degeneration of the pathway. 200X mag. Middle panels – Synaptic zinc staining reveals the hTau-induced partial loss of lateral perforant pathway synapses in the OML (compare the injected with the uninjected side). 200X mag. Bottom panels - NeuN staining of the lateral EC reveals hTau-driven degeneration of layer II neurons of origin for the lateral perforant pathway (just right of the asterisks). 100X mag.

7. Mild TBI does not modify the expression level, subcellular distribution or synaptic spread of human tau or hyperphosphorylated tau chronically post-injury.

With our AAV model for localizing human tau expression to the mouse lateral perforant pathway, we are well positioned to determine whether mTBI exacerbates tau pathology chronically after injury. We reported previously that, in the acute post-injury period, neither sham-, single, nor double mTBI cases show human tau expression beyond the lateral perforant pathway. We now report similar findings at 4 months post-injury. At 4 months after single (data not shown) or repetitive mTBI (Figure 7), total human tau expression remains restricted to the lateral perforant pathway neurons of origin in layer II of lateral entorhinal cortex, the perforant pathway axons in the distal hippocampal CA1 sector, the SLM, and to their synaptic field in the dentate gyrus OML (Figure 7). The rest of the hippocampal formation is devoid of human tau, including the neuronal targets for the lateral perforant pathway, the dentate granule neurons in the GCL. Quantitative analysis of human tau spread into the dentate granule neuron targets for the lateral perforant pathway will be completed during the EWOF period. The findings to date suggest that, as was true acutely post-injury, mTBI does not induce any large scale spread of pathological human tau from the perforant pathway in the controlled cortical impact mouse model in the chronic post-injury period.

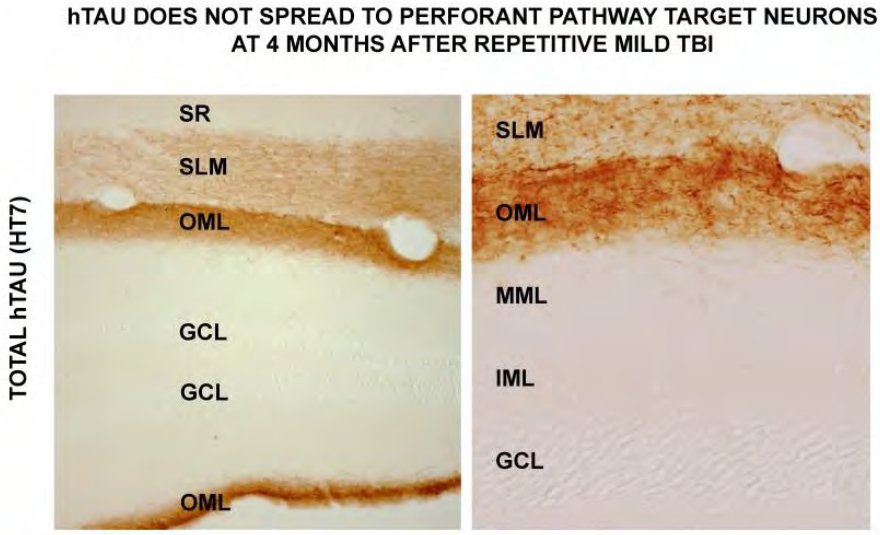


Figure 7. In the chronic post-mTBI time period, human tau expression remains confined to the lateral perforant pathway axons (SLM) and synaptic field (OML), and does not spread to the dentate granule target neurons (GCL) or any other hippocampal cell type. This is true for both blades of the dentate gyrus (left), and for all 3 injury groups.

In addition to the lack of effect of mTBI on the cellular distribution of human tau, there is no consistent change in hTau expressing mice in the degree of tau phosphorylation, as evidenced by comparing the immunostaining intensities for total human tau (labeled with the HT7 monoclonal), pTau202/205 (AT8 monoclonal), and pTau231 (PHF6 monoclonal) between the sham-injured, mTBI, and double mTBI groups (data not shown). Furthermore, endogenous mouse tau does not undergo hyperphosphorylation chronically after single or double mTBI, as evidenced by the absence of pTau202/205 or pTau231 in either entorhinal cortex or dentate gyrus at 4 months post-injury in mice expressing eGFP in the lateral perforant pathway. In summary, in the chronic post-injury period neither single nor double mTBI changes the phosphorylation of human or endogenous mouse tau, the expression level of human tau, its subcellular compartmentation, or its cellular localization.

8. Evidence for slowly progressive tauopathy and tau-mediated perforant pathway neuronal degeneration in our mouse model, irrespective of mild traumatic brain injury.

8A. A subset of tau phosphoforms become mislocalized in perforant pathway neurons.

Using immunohistochemistry with antibodies specific for several phosphorylated forms of tau, pTau202/205, pTau231, pTau212/214/217, and pTau262, we showed previously that, at 5 weeks after AAV-hTauP301L delivery, phospho-tau was restricted to the neurons of origin for the lateral perforant pathway in layer II of the lateral entorhinal cortex (Figure 8). Each tau phosphoform showed diffuse and uniform distribution in the cytoplasm of neuronal perikarya. These phospho-tau positive neurons have the morphology of healthy pyramidal neurons. Phospho-tau localizations were not affected by either single or double mTBI at one week post-injury, as described in prior reports. However, at 5 months after AAV-hTauP301L gene delivery, distribution of specific phospho-tau variants shows profound changes from the 5 week time point. Whereas pTau202/205 and pTau231 remain uniformly distributed within most healthy-looking neurons, scattered neurons also exhibit punctate perikayal pTau202/205 staining. Most strikingly after 5 months of pathological human tau expression, occasional neurons no longer show the diffuse cytoplasmic localization of pTau262 and pTau212/214/217, but instead develop focal cytoplasmic inclusions strongly immunopositive for these two tau phosphoforms.

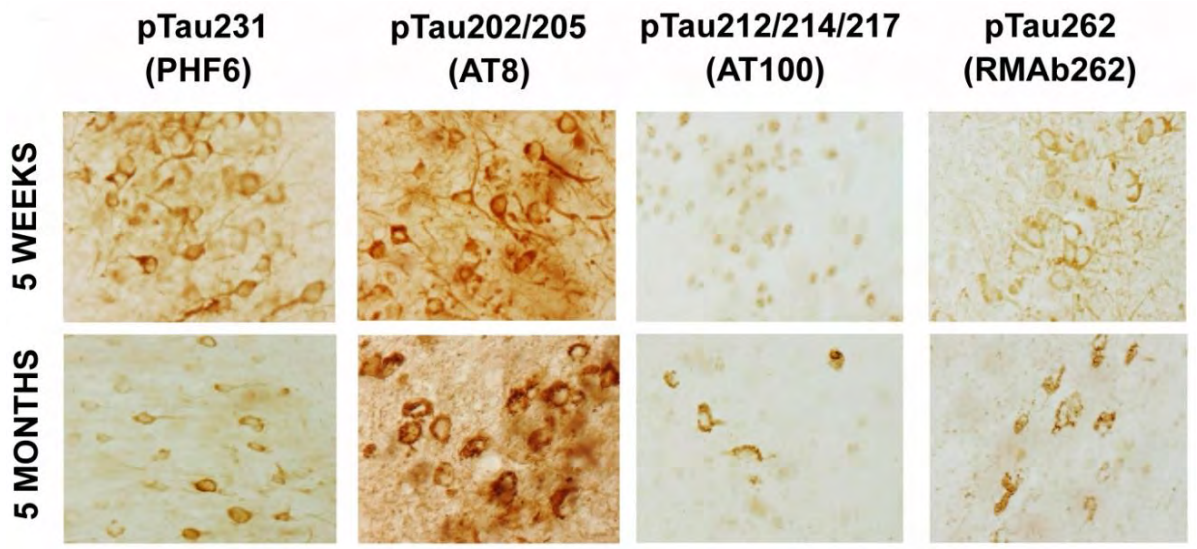


Figure 8. Evidence for slowly progressive accumulation of specific tau phosphoforms in scattered neurons of the lateral perforant pathway at 5 months after hTauP301L gene delivery.
Lateral entorhinal cortex at either 5 weeks or 5 months after hTauP301L gene delivery
(LEFT 4 PANELS) – Acutely post-mTBI, both pTau231 and pTau202/205 are diffusely distributed throughout the cytoplasm of layer II entorhinal neurons. Distribution of the former is unchanged at 5 months, whereas some of the pTau202/205 accumulates within cytoplasmic puncta.
(RIGHT 4 PANELS) – There is little appreciable pTau212/214/217 whereas pTau262 shows diffuse cytoplasmic distribution at 5 weeks after gene delivery By 5 months, however, these two tau phosphoforms accumulate into puncta, often associated with cytoplasmic vacuoles.

8B. The mislocalized tau phosphoforms are associated with degenerating perforant pathway neurons.

At 5 months after AAV-hTauP301L gene delivery, a subset of perforant pathway neurons with phospho-tau expression show profound morphological changes compared to the 5 week time point (Figure 9). Whereas pTau202/205 and pTau231 remain relatively uniformly distributed within most neurons with healthy morphology, sparse neurons exhibit intense pTau202/205 staining with abnormal round perikarya (e.g., left panel, arrow). The neurons with strong accumulation of pTau202/205 also contain this phospho-tau form in dendrites with beaded varicosities typical of ongoing dendritic degeneration (second panel from left, arrowheads). Phospho-tau accumulation within degenerating perforant pathway neurons is even more pronounced for the pTau262 form (right two panels). Intense pTau262 is found in neurons with abnormally shaped cell bodies (far right panel) and beaded dendritic varicosities (arrowheads). The long-term accumulation of this subset of tau phosphoforms in scattered, actively degenerating lateral perforant pathway neuronal perikarya and dendrites occurs independently of mTBI. As shown in Figure 9, at 5 months after gene delivery of pathological human tau, the p202/205 and p262 tau phosphoforms accumulate abnormally within the cell bodies of some perforant pathway neurons in layer II of the lateral entorhinal cortex even in sham-injured mice. Taken together, these data suggest that expression of a low dose of pathological human tau in the mouse lateral perforant pathway, below the threshold for eliciting rapid degeneration of the pathway, instead evokes a slowly progressive tau-mediated neurodegeneration. This tau proteotoxicity occurs independently of mTBI, and is preferentially associated with tau phosphorylation on residues 212, 214, 217, and 262.

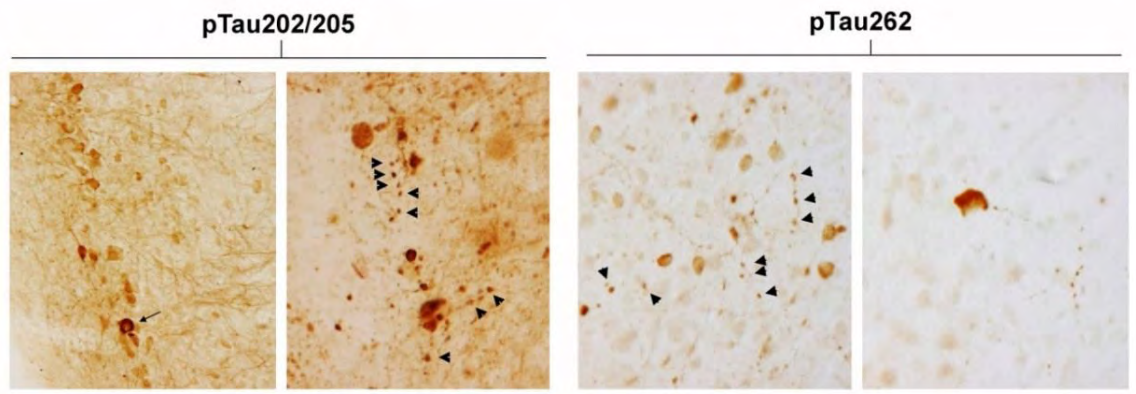


Figure 9. Tau phosphoforms p202/205 and p262accumulate in scattered perforant pathway neurons with degenerating processes after 5 months of pathological human tau expression in sham-injured mice.

Immunostaining of lateral perforant pathway neurons shows a subset express relatively high amounts of pTau202/205 (left panel, arrow) and pTau262 (far right panel) at 5 months after hTauP301L gene delivery. The neurons accumulating these two phospho-tau forms have rounded cell bodies and beaded dendrites (arrowheads), morphology indicating active neurodegeneration. The onset of phospho-tau triggered degeneration is independent of mTBI, as it occurs in sham-injured controls.

8C. Certain phospho-tau variants accumulate in granulovacuolar degeneration bodies.

Another pathological feature of some lateral perforant pathway neurons observed after 5 months but not 5 weeks of hTauP301L expression is the strong concentration of specific tau phosphoforms within cytoplasmic granules. The granules resemble pathological inclusions that have been termed granulovacuolar degeneration (GVD) bodies, which are known to accumulate intraneuronally in afflicted brain regions in Alzheimer's and other chronic neurodegenerative diseases (reviewed by Kohler, 2016). After 5 months but not 5 weeks of hTauP301L expression, some pTau212/214/217 and pTau202/205 becomes concentrated in cytoplasmic inclusions resembling GVD bodies, and no longer shows uniform diffuse cytoplasmic distribution (Figure 10). Most strikingly, pTau262 is found in these scattered neurons exclusively in presumptive GVD bodies after 5 months of human tauP301L expression. This is the time when some perforant pathway neurons develop abnormal neurodegenerative morphologies (Figure 9), suggesting the pTau granule formation and neurotoxicity may be related phenomena. The histological similarity of focal granules of pTau262 to the GVD bodies in neurons of the Alzheimer's brain is illustrated by GVD immunolabeling in the bottom right panel by the marker casein kinase I (Kohler, 2016). The evidence linking the specific tau phosphoform pTau262 to GVD bodies and slowly progressive neurodegeneration has implications for understanding the molecular and cellular processes by which tau triggers neurotoxicity and drives regional brain atrophy in Alzheimer's disease.

9. mTBI impairs hippocampus-dependent spatial learning: no robust chronic effect of pathological human tau.

In addition to the array of histopathological methods for evaluating pathological tau and mTBI-induced long-term changes in hippocampal structure following TBI, we completed analysis of long-term post-injury hippocampal function this past year. We used a Morris Water Maze to assess spatial learning over 3 consecutive days beginning 4 months after single or double mTBI or sham injury (5 months after AAV-mediated gene delivery). Our cognitive test data indicate that both single and double mTBI cause subtle impairments in spatial learning

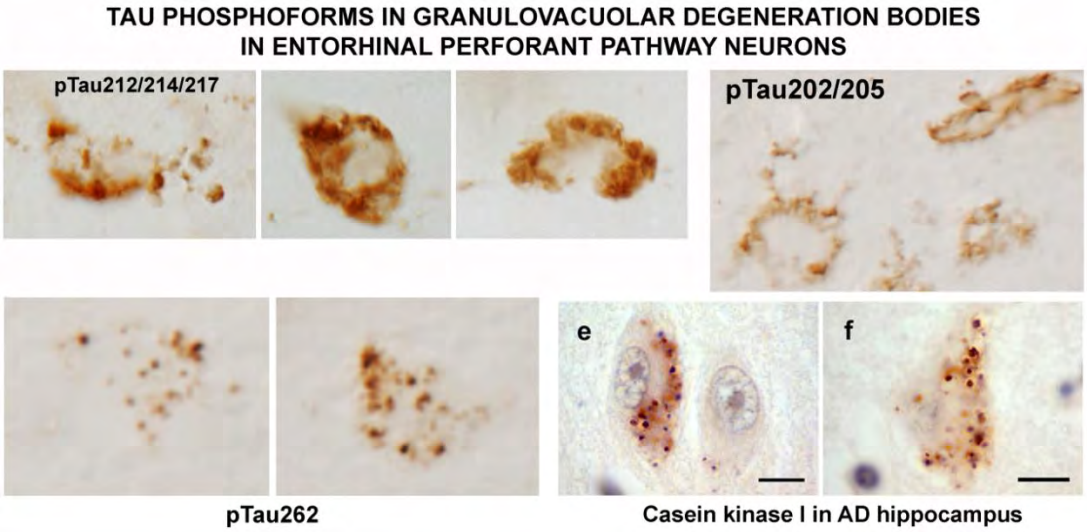


Figure 10. High power light microscopic assessment of cytoplasmic phospho-tau granules in mouse perforant pathway neurons developing 5 months after expression of hTauP301L.
Top left – pTau212/214/217 accumulates in cytoplasmic puncta surrounding the unstained nucleus.
Top right – pTau202/205. Like pTau212/214/217, this tau phosphoform remains diffuse in cytoplasm but mostly concentrates within granules.
Bottom left – Unlike other tau phosphoforms, pTau262 is localized exclusively to cytoplasmic inclusions with the appearance of GVD bodies.
Bottom right – From Kohler (2016). GVD bodies in neurons of the human AD brain immunostained for the marker casein kinase I highlights their similarity to the mouse brain granules with pTau262.

during the chronic post-injury time period, and unilateral human tau expression in the perforant pathway does not appreciably exacerbate the learning deficits when compared with eGFP expression as a control foreign protein. Unilateral human tauP301L by itself, in the absence of mTBI, shows a trend to impair spatial learning after 5 months of transgene expression, but this effect does not reach statistical significance.

Figure 11 illustrates the data compilation on swim latency of mice to find the hidden platform using visual cues as a measure of spatial learning. It shows that mice expressing either eGFP or hTauP301L and tested 4 months after sham surgery learn with repeated training to locate the hidden platform, based on statistically significant decreases in latency on the third day of training compared with the initial training day. There is a non-significant trend for greater improvement with training in the eGFP sham-injured group than the hTauP301L mice ($p=0.10$). Chronically after single or double mTBI, eGFP expressing mice subjected to single mTBI exhibit longer latencies than sham-injured mice, a trend toward impaired spatial learning ability that did not reach statistical significance ($p<.06$). The sham-injured hTau expressing mice also exhibit spatial learning based on a significant decrease in swim latency from the first to the third day of training. Whereas no significant improvement with training was exhibited by the hTau single mTBI group, after double mTBI the hTau group showed significant improvement in swim latency on training day 3. Finally, there were no significant differences between eGFP- and hTau-expressing mice after either single or double mTBI on any day of training, indicating that unilateral expression of hTauP301L in the lateral perforant pathway does not appreciably worsen the mTBI-induced spatial learning deficit chronically post-injury.

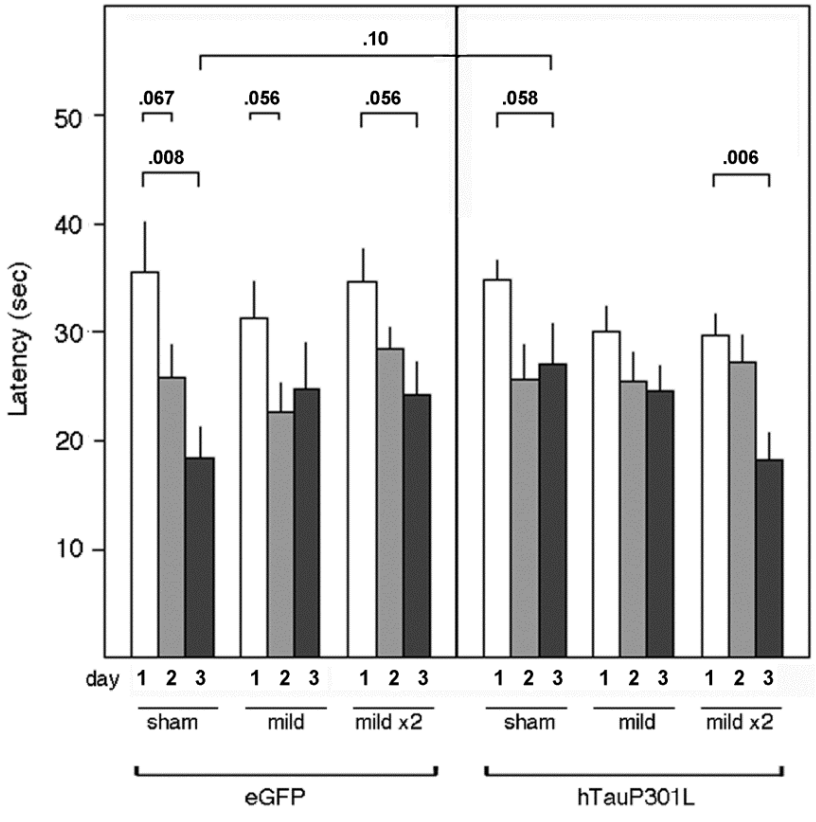


Figure 11. mTBI subtly impairs spatial learning chronically on the Morris Water Maze, an effect of injury that is not exacerbated by perforant pathway hTauP301L expression. The mean swim time latency (in seconds+s.e.m.) is shown on three consecutive days of training (6 trials/day) at the indicated days starting 4 months after mice received sham injury, mTBI, or double mTBI. Both single and double mTBI caused learning deficits for the eGFP-expressing mice, and double injured hTau-expressing mice. It is noteworthy that there were no significant differences between groups expressing hTauP301L or eGFP in the lateral perforant pathway on any day of training or with any type of injury, although long-term expression of hTauP301L caused a non-significant learning impairment in the absence of mTBI (p=0.10). (Shown along the top are P values, unpaired t-test).

From the collective data for both the acute and chronic post-injury phases, we conclude that (i) mice exhibit spatial learning with repeated training on consecutive days; (ii) both single and double mTBI cause subtle but discernable impairments in spatial learning both acutely and chronically post-injury; and (iii) unilateral expression of hTauP301L in the lateral perforant pathway does not worsen the deleterious effects of single or repetitive mTBI on spatial learning. Finally, our data suggest that long-term expression of pathological human tau in the lateral perforant pathway may subtly impair spatial learning in the absence of any brain injury, although this trend did not reach statistical significance. The collective behavioral data from the past 3 years indicate that pathological human tau has no worsening effect in the acute or chornic post-injury periods on hippocampus-dependent spatial learning, a conclusion fully consistent with the lack of overt effect of pathological human tau on perforant pathway structural integrity after single or double mTBI.

10. Blood-based biomarkers for mild TBI-induced brain damage: Serum SNTF and hypophosphorylated neurofilament H await evaluation at 4 months post-injury.

In year two of this project, we analyzed at 7 days post-injury the serum level of a protein biomarker for TBI-induced axonal damage discovered and characterized previously by the Siman laboratory, the calpain-derived α-spectrin N-terminal fragment SNTF (α-spectrin 1-1176). SNTF levels rise in the blood on the day of human concussion treated in the emergency room, and are elevated preferentially in those cases exhibiting white matter abnormalities detected by advanced neuroimaging, as well as persisting cognitive performance problems. In professional ice hockey players, serum SNTF increases after an in-game concussion preferentially in players that will go on to develop persisting post-concussion symptoms affecting their return to play. Histopathological studies demonstrate that this calpain derivative normally is below the limit of detection in the brain, but accumulates within damaged axons after TBI in humans and mTBI in a large animal experimental model of concussion (Johnson et al., Acta Neuropathol. *131*, 115 [2016]). On these and other bases, SNTF has emerged as the lead blood biomarker for the prognosis of concussion, and its elevation is a biologically plausible surrogate marker for diffuse axonal injury.

Given the lack of appreciable change in serum SNTF at 7 days post-injury in the mouse we reported previously, our study is poised to determine whether this biomarker for diffuse axonal injury increases preferentially in the long-term post-injury period as a surrogate marker for a chronic neurodegenerative condition, and whether pathological human tau expression in the perforant pathway worsens any long-term effect of mTBI to elevate this surrogate biomarker for progressive brain damage. In addition, our laboratory has discovered that a hypophosphorylated form of the cytoskeletal protein neurofilament H also is elevated in the blood following acute brain damage, and this neuron-specific protein merits consideration as a potential serum biomarker for chronic, progressive mTBI-induced brain injury in the mouse model. The analyses of these two markers will be completed during the Extension Without Funding period.

11. Summary of major findings:

Milestones I and II: (i) determine whether tau worsens hippocampal structure and function in the acute and long-term post-TBI time periods; (ii) determine whether mTBI worsens incipient tauopathy acutely and chronically after injury; (iii) determine whether blood levels of neurodegeneration biomarkers measured at 7 days and 4 months post-injury correlate with the severity of brain damage after mTBI.

Taken together, our data from the past three years indicate that pathological human tau expressed in a major hippocampal input pathway does not appreciably worsen TBI-induced changes in the structure of the pathway, or in pathway-dependent cognitive function in either the acute or chronic post-injury periods. This holds true for both single and double mild TBI. Our data also show that neither single nor double mTBI promote tauopathy by worsening tau hyperphosphorylation or promoting its aggregation and anatomical spread at either 7 days or 4 months post-injury.

How do these conclusions, that no discernable interaction occurs in the mouse brain between pathological human tau and single or double mTBI in the acute and long-term post-injury periods, relate to current literature on the role of tau pathology in human TBI? They are largely consistent with recent reports. The histopathological study of post-mortem human brains obtained from a few days to one month after TBI reported no change in neuronal expression or phosphorylation of tau in the sub-acute post-injury time frame, quite similar to our observations

in our mouse-based project. Whereas retrospective epidemiological studies initially suggested that TBI could increase the risk of developing Alzheimer's disease later in life, recent studies with improved designs have provided evidence that brain dysfunction developing long after mild TBI affects cognitive domains distinct from those especially impacted in Alzheimer's disease. Moreover, studies of elderly war veterans have failed to confirm an increased risk of Alzheimer's disease in the long-term period after mild TBI (Seal et al., 2016; Weiner et al., 2017). Coupling our findings reported herein using a well-controlled mouse experimental model with recent human mild TBI studies, there is no compelling evidence that tau pathology represents a key pathogenic event after mild TBI, and little support that tau pathology is a viable therapeutic target for mitigating the long-term effects of mild TBI.

During year 3 of the project, we completed most of the planned evaluations of interactions between tauopathy and mTBI in the long-term post-injury period. In addition, we established a bank of serum samples obtained at the time of sacrifice at 4 months post-injury for the study of biomarkers that may predict the development of a TBI-induced chronic neurodegenerative condition in the long-term post-injury period. These serum biomarker analyses along with remaining quantitative histopathological assessments of the effects of mild TBI coupled with pathological human tau expression on synapse loss and tau trans-synaptic spread will be completed during the recently granted EWOF period, extended to March 29, 2018.

Impact

Mild traumatic brain injury (mTBI) is the most common neurological injury in civilians, and affects over 1.5 million children and adults each year in the United States. Although mTBI is typically undetectable with computed tomography, it can elicit long-term and clinically significant brain dysfunction in ~25% of cases. At the present time, there are neither methods that can identify at an early and treatable stage the subset of mTBI sufferers who will go on to develop acute brain damage and long-term disability, nor clinically proven treatments for improving brain functional outcome. Consequently, new approaches are urgently needed for rapidly identifying mTBI patients in the acute post-injury period who are at risk of suffering persistent brain dysfunction, and for treating these at-risk cases to preserve brain structure and function. Furthermore, accumulating evidence suggests that both single and repetitive TBIs can lead in later life to a chronic, progressive Alzheimer's disease (AD)-like neurodegenerative disorder. New methods are needed to identify those individuals that are beginning to develop TBI-triggered chronic neurodegenerative disease, and new treatments urgently need to be developed for slowing their chronically progressive brain atrophy and cognitive decline.

In the long-term post-injury time period, mTBI shares neuropathological features with AD. Moreover, given that AD is a slowly progressive neurodegenerative and cognitive disorder, and TBI will sometimes lead to chronic progressive brain atrophy and cognitive decline, the question arises whether AD and TBI may share common underlying pathophysiology. One of the pathological hallmarks of AD is the aggregation of the protein tau into neurofibrillary tangles within vulnerable neurons in brain regions important for higher cognitive function. Considerable evidence implicates tau pathology as a key pathogenic driving force for the progressive brain atrophy and inexorable cognitive decline. On the other hand, whereas mTBI will also sometimes cause tau abnormalities that superficially resemble the tauopathy of AD, the pathophysiological roles for tau in the acute and chronic periods after TBI are unknown. Here, we are examining directly and critically whether tauopathy plays important roles in the acute and chronic outcomes from single and repetitive mTBI. Our study evaluates in a well-controlled pre-clinical experimental model the interrelationships between TBI and subsequent AD, thereby fostering discovery of new therapeutic strategies for military personnel, veterans, and civilians exposed to single or repetitive mTBI. *Based on the histopathological and neurocognitive responses to single and double mTBI, our data indicate that the presence of a pathological form of human tau in the mouse perforant pathway does not render the neurons, axons, or synapses of this projection more vulnerable to single or double mTBI in either the acute or chronic post-injury periods. In addition, neither single nor double mTBI exacerbate ongoing tauopathy either acutely or chronically after mTBI. Our data do not support aspects of tauopathy as being viable therapeutic targets for mitigating the acute or chronic effects of mild TBI.*

Finally, our study has been evaluating pre-clinically new diagnostic and prognostic blood tests for identifying at early and treatable stages the subsets of mTBI cases at risk of developing brain damage and long-term dysfunction. Simple blood tests for TBI induced brain damage are vitally needed, and would have major applications for both military and civilian sufferers of mTBI. *Thus far, serum levels of the neurodegeneration biomarker SNTF are not appreciably elevated at 7 days post-TBI. During the recently granted EWOF period, serum SNTF and another candidate neurodegeneration biomarker will be analyzed as possible surrogate blood measures for chronic neurodegenerative changes induced by mTBI developing only in the long-term post-injury period.*

Changes/Problems

No significant changes in study design or conduct were introduced during the third year of the project.

Products

Nothing to report. Two manuscripts are in preparation detailing (i) the lack of interaction between tauopathy and mild TBI in the acute and chronic post-injury periods, and (ii) the development of slowly progressive neurodegeneration potentially driven by specific tau phosphoforms and cell biological processes in a neural circuit that is especially vulnerable at an early stage in Alzheimer's disease.

Participants and other support

1. Dr. Robert Siman
   Role –Principal Investigator
   Effort – 2.4 person months/year 3
   Contribution – Dr. Siman has directed every aspect of the project. He formulated the experimental strategies. He trained personnel on the requisite methods of stereotaxic neurosurgical viral vector-based gene delivery, animal husbandry, histology, immunohistochemistry, microscopy, quantitative morphometry, and serum preparation. He assisted with histological assessments of perforant pathway neuronal, axonal, and synaptic integrity after traumatic brain injury. He performed photomicroscopic documentation of the research findings thus far, and prepared all quarterly and annual reports. He validated the immunoassays for neurodegeneration biomarkers using new equipment purchased through this award.

1. Dr. Victoria Johnson
   Role – Co-Investigator
   Effort – 1.2 person months/year 3
   Contribution – Dr. Johnson has performed the controlled cortical impact traumatic brain injuries and sham injuries, and has introduced methodological improvements to enhance the precision and consistency with which the injury device elicits mild TBI. She ensured personnel were trained thoroughly on the evaluation of spatial learning using the Morris Water Maze task. She assisted with histological study of mTBI-induced axonal pathology.

2. Ms. Hongmei Cui
   Role – Research Specialist, Siman laboratory
   Effort – 3 person months/year 3
   Contribution –In year 3, Ms. Cui performed all of the neurosurgical methods and serum biomarker analyses conducted thus far on the short- and long-term (4 month) outcomes from single and double mTBI.

3. Mr. Samuel Feintech
   Role – Research Specialist, Siman laboratory
   Effort – 6 person months/year 3

In year 3, Mr. Feintech conducted the qualitative and quantitative histopathological evaluations of tau expression and pathology, neuronal and synapse integrity, and axonal vulnerability of the genetically modified perforant pathway at 4 months after single or repetitive mTBI. In April, 2017, he received training in the Morris Water Maze task of spatial learning, and completed the neurobehavioral evaluations of the final batches of mice in the long-term post-injury period.

Other support

"Investigating the neurologic effects of training associated blast"
DARPA award NEU-92-2913
Joshua Duckworth, Principal Investigator
Robert Siman, Co-Investigator (1.8 calendar months effort)
Period: 7/1/16 – 6/30/19
Administration: Henry M. Jackson Foundation
Subaward Specialist: Alison Dineen, adineen@hjf.org
Annual direct cost (Siman sub-contract): $69,000
Overlap: There is no overlap with the current project. This award funds a human research study into the effects of heavy weapons training in the military on brain injury and functional status. Dr. Siman is assessing a set of blood biomarkers for neurodegeneration as potential surrogate markers for training-induced brain injury. Longitudinal serum and plasma levels of SNTF and hypophosphorylated Neurofilament H are being compared with neuroradiological and neurobehavioral evaluations of training-induced brain structural and functional changes, and with cumulative blast exposures.

"Diagnosis and mechanisms of mild traumatic brain injury"
NIH R01NS092398-01
Douglas Smith, Principal Investigator
Robert Siman, Co-Investigator (0.6 calendar months effort)
Period: 4/1/15 – 3/31/20
Administration: National Institute of Neurologic Disorders and Stroke
Annual Direct Cost (Siman laboratory): $20,000
Overlap: None. The project is focused on mechanisms and biomarkers for diffuse axonal injury in a large animal model of concussion.

"Diffuse and focal brain injury in a large animal model of post-traumatic epilepsy: Mechanisms underlying epileptogenesis"
Source: Department of Defense award W81XWH-15-ERP-IDA
John A Wolf, Principal Investigator
Robert Siman, Co-Investigator (0.6 months calendar effort)
Period: 9/30/2016 – 9/29/2019
Administration: Department of Defense
Award Specialist:
Annual direct cost (Siman laboratory): $9,000
Overlap: None. The project studies the comparative contributions of diffuse and focal brain injury to the development of epileptogenesis in two large animal models of traumatic brain injury, and seeks discovery of a blood biomarker predictive of post-traumatic epilepsy.

Special Reporting Requirements

Not applicable.

Appendix

None to report.